BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

STIC-ILL

From: Canella, Karen
Sent: Wednesday, May 14, 2003 3:05 PM
To: STIC-ILL
Subject: ill order 09/230,955

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/230,955

1. American Journal of Pathology:
   1993 Feb, 142(2):403-412
   1993, 143(4):1150-1158
   1984, 114(3):454-460
   1996, 148(3):865-875
   1965 Sep, Vol. 44, pp. 280-282

2. Cancer Research, 1993 May 15, 53(10 suppl):2287-2299

3. Cancer epidemiology, biomarkers and Prevention, 1996 Jul, 5(7):549-557

4. Lab Investigation:
   1980, 42(1):91-96
   1988, 58(2):141-149

5. Gynecol Oncol, 1982, 13(1):58-66

6. International Journal of Gynecological Pathology:
   1985, 4(4):300-313
   1986, 5(2):151-162
   1992, 11(1):24-29

7. Differentiation:
   1986, 31(3):191-205
   1988, 39(3):185-196

8. Cancer (Phila), 1989, 63(7):1337-1342

9. Cancer Res, 1990, 50(16):5143-5152

10. Virchows Arch B Cell Pathol Incl Mol Pathol, 1987, 54 (2):98-110

11. Acta Histochemica et Cytochemica:
    1994, 27(3):251-257
    1996, 29(1):51-56

12. Archives of Gynecology and Obstetrics, 1989, 246(4):233-242

13. Clin Lab Med, 1995 Sep, 15(3):727-742

14. Clin Obstet Gynaecol, 1984 Apr, 11 (1):5-23

Detection of Squamous Cell Carcinoma Antigen in Normal Squamous Epithelia and in Squamous Cell Carcinomas of the Uterine Cervix

GERD CROMBACH, MD,* ANTON SCHARL, MD,* MATTHIAS VIERBUCHEN, MD,† HANNELORE WÜRZ, PHD,* AND ACHIM BOLTE, MD*

Squamous cell carcinoma (SCC) antigen is a subfraction of tumor antigen TA-4 isolated from a cervical squamous cell carcinoma. The specificity of SCC antigen and the factors influencing its release into serum were evaluated. Antigen concentrations were measured in 157 tissue extracts and in 188 sera of patients with nonmalignant or malignant gynecologic diseases. A commercial radioimmunoassay based on polyclonal antibodies (Abbott Laboratories, North Chicago) was used. Cytosol concentrations were significantly higher ($P < 0.005$) in normal squamous epithelia ($\tilde{x}$ = 6040 ng/mg cell protein [CP]) and in squamous cell carcinomas ($\tilde{x}$ = 2483 ng/mg CP) of the exocervix than those in normal columnar epithelia and in adenocarcinomas of the endocervix, endometrium, ovary, and breast ($\tilde{x}$ = 1–508 ng/mg CP). Despite the high antigen concentrations in normal squamous epithelia, elevated serum levels (>2.5 ng/ml) were almost exclusively found in patients with cervical squamous cell carcinomas. The sensitivity of SCC antigen as a marker for primary carcinomas was 61%, increasing from 29% in Stage I to 89% in Stage IV. The positivity rate was higher in women with well-differentiated (78%) and moderately differentiated carcinomas (67%) than in those with poorly differentiated tumors (38%). The results show that SCC antigen is not tumor specific. The release into serum is independent of local tissue content, but is apparently influenced by the infiltrative growth, the mass, and the degree of histologic differentiation of the tumor.

Cancer 63:1337–1342, 1989.

In 1977, Kato and Torigoe isolated the tumor antigen TA-4 from a cervical squamous cell carcinoma.[1] TA-4 is a glycoprotein with a molecular size of 48.000 daltons which is located in the cytoplasm of normal squamous epithelia and squamous cell carcinomas of the uterine cervix.[2,3] Squamous cell carcinoma (SCC) antigen is one of 14 subfractions of TA-4, purified from liver metastases of a cervical squamous cell carcinoma.[4] The clinical value of TA-4 and SCC antigen as serum tumor markers for cervical cancer has been demonstrated in numerous studies.[2,5–15] The sensitivity is 44% to 67% in primary and 67% to 100% in recurrent cervical squamous cell carcinomas. Serum levels of both antigens reflect the extent of the carcinoma and the progression or regression of the tumor during follow-up. However, neither antigen is specific for cervical squamous cell carcinomas. Elevated serum levels are also found in 24% to 53% of patients with squamous cell carcinomas of the head and neck, esophagus and lung,[5,9,16–18] and in 8% to 42% of patients with adenocarcinomas of the uterus, ovary, and lung.[13,16,17]

Until now, detailed studies on the distribution of SCC antigen in different body tissues have not been published, probably due to the lack of a suitable antibody for immunohistochemical analysis. The current study evaluates the specificity of SCC antigen and the factors influencing the release of the antigen into the circulation. Antigen concentrations were simultaneously measured in tissue extracts and sera of patients with normal epithelia or different carcinomas of the female genital tract. Serum concentrations of SCC antigen in patients with cervical squamous cell carcinomas were correlated to the clinical stage and to the degree of histologic differentiation of the tumors.

Materials and Methods

One hundred fifty-seven tissue specimens were obtained from 108 patients undergoing surgery for nonmalignant gynecologic disorders (myoma, endometriosis, descensus, fibroadenoma) (n = 45) or for malignant gynecologic tumors (n = 63). In 21 of the 45 women with benign lesions, tissue sections were simultaneously prepared from two or

From the Departments of *Obstetrics and Gynecology and †Pathology, University of Cologne, Federal Republic of Germany.

The authors thank Doris Peters for technical assistance.

Address for reprints: Gerd Crombach, MD, Universitäts-Frauenklinik Köln, Kerpener Str. 34, D-5000 Köln 41, Federal Republic of Germany.

Accepted for publication October 24, 1988.

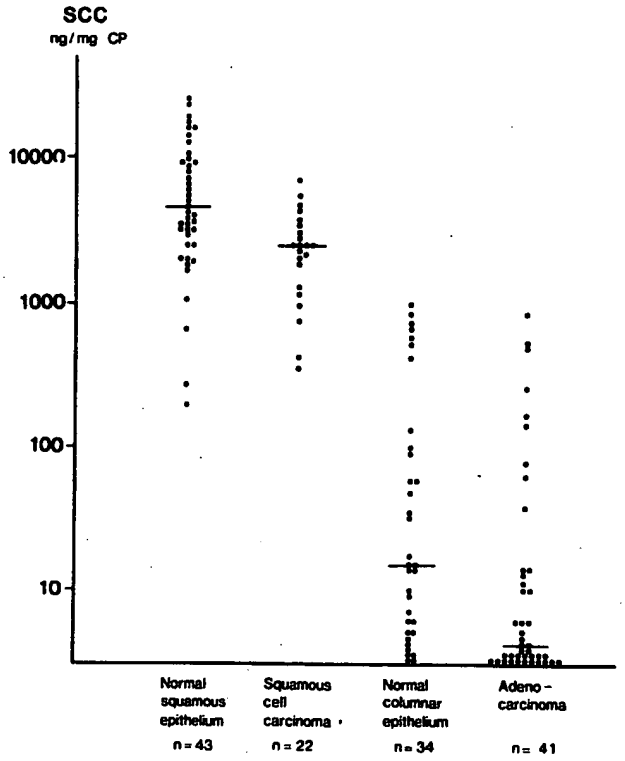

FIG. 1. Squamous cell carcinoma antigen concentrations (ng/mg cell protein) in the cytosol of normal epithelia and carcinomas of the vulva, vagina, uterine cervix, endometrium, ovary, and breast (—: median value).

more sites of the genital tract. The following biopsy specimens were taken: (1) normal squamous epithelia of the exocervix (n = 31), vagina (n = 7), and breast skin (n = 5); (2) normal glandular epithelia of the endocervix (n = 17) and endometrium (n = 17); (3) normal stroma of the cervical wall (n = 17); (4) squamous cell carcinomas of the uterine cervix (n = 15), vulva (n = 5), and vagina (n = 2); and (5) adenocarcinomas of the endocervix (n = 4), endometrium (n = 17), ovary (n = 10), and breast (n = 10).

Tissue specimens were divided into two parts for histologic examination and for cytosol preparation. Fresh biopsy specimens were cooled on ice immediately after excision, cut and weighed, pulverized under liquid nitrogen (using a microdismembrator, obtained from Braun-Melsungen, FRG), extracted with a four-fold amount of phosphate buffer (0.01 mol/l, pH 7, containing 10% of glycerol) and centrifuged for 45 minutes at 100000 × *g*.

The cytosol was separated from the pellet and the lipid layer. The total protein content was determined according to Lowry *et al.*[19] and was corrected for serum albumin contamination by radial immunodiffusion (using M-Partigen plates from Behringwerke, Marburg, FRG). Cytosol specimens were stored at −70°C until used. A volume of 0.1 ml pure or diluted cytosol was used for each assay.

Serum samples were obtained from 82 of the above-mentioned 108 patients, from 93 women with primary squamous cell carcinomas and from 13 patients with recurrent squamous cell carcinomas of the uterine cervix. All sera were stored at −20°C until used. Diagnosis was confirmed by histologic examination in all cases. Clinical staging of primary cervical squamous cell carcinomas was based on the criteria of the International Federation of Gynecologists and Obstetricians (FIGO). Cervical squamous cell carcinomas were classified as well-differentiated (Grade 1), moderately differentiated (Grade 2), and poorly differentiated (Grade 3) tumors. Criteria of classification were the polymorphism of cells and nuclei, nucleus/cytoplasm ratio, mitotic activity, and keratin formation.[20]

The SCC concentrations in the cytosol (ng/mg cell protein [CP]) and serum (ng/ml) were determined by a double-antibody radioimmunoassay (Abbott-Diagnostics, Wiesbaden, FRG), based on the reactivity of a polyclonal goat antibody. The limit of the normal range in serum corresponded to the threshold value of 95% specificity in healthy female controls and was 2.5 ng/ml.[13] The coefficients of intraassay and interassay variation were 13.8% and 15.0%, respectively.

The Mann-Whitney test was applied for evaluation of significant differences between individual groups of tissue specimens or serum samples. Differences were considered significant when the probability of error was below 5% ($P < 0.05$).

Results

The distribution of SCC antigen concentrations in the cytosol of normal epithelia of the female genital tract and of gynecologic carcinomas is shown in Figure 1. The antigen values ranged from 194 to 25.033 ng/mg CP in normal squamous epithelia, from 350 to 6917 ng/mg CP in squamous cell carcinomas, from 1 to 975 ng/mg CP in normal columnar epithelia, and from 1 to 850 ng/mg CP in adenocarcinomas. The median values in these groups were 4529, 2477, 15, and 3 ng/mg CP, respectively. The antigen concentrations in normal squamous epithelia and in squamous cell carcinomas were significantly higher than those in normal columnar epithelia and in adenocarcinomas ($P < 0.00001$). The SCC antigen values exceeding 1000 ng/mg CP were measured in 40 of 43 normal squamous epithelia and in 18 of 22 squamous cell carcinomas, but in none of 34 normal columnar epithelia or of 41 adenocarcinomas.

The highest SCC antigen levels were found in the cytosol of normal squamous epithelia of the exocervix (Table 1). In nine of 31 cases the values lay above 10.000 ng/mg

TABLE 1. Squamous Cell Carcinoma Antigen Concentrations in the Cytosol of Normal Epithelium and Stroma (ng/mg Cell Protein) and in Serum (ng/ml)

	Cytosol			Serum		
	n	Range (ng/mg CP)	Median (ng/mg CP)	Elevated levels (>2.5 ng/ml)	Range (ng/ml)	Median (ng/ml)
Squamous epithelium						
Exocervix	31	194–25,033	6040	2/19	0.3–5.6	1.7
Vagina	7	2008–8671	3613	1/6	0.3–5.6	1.6
Breast skin	5	269–6060	2520	0/4	0.3–2.0	0.8
Columnar epithelium						
Endocervix	17	4–975	97	1/12	1.2–3.3	1.9
Endometrium	17	1–57	6	1/12	1.2–3.3	1.9
Stroma						
Cervical wall	17	4–409	22	1/12	1.2–3.3	1.9

CP: cell protein.

CP. The median concentration in this group was 2.3-fold higher than that in squamous cell carcinomas of the cervix (Table 2). The antigen values of these groups were significantly different ($P < 0.0005$). Despite the extremely high antigen concentrations in normal squamous portio epithelia, only two of 19 patients had increased SCC antigen levels in serum (3.3 and 5.6 ng/ml, respectively). In contrast, women with cervical squamous cell carcinomas had lower cytosol concentrations, but higher antigen values in serum (Tables 1 and 2). Elevated serum levels up to 61.9 ng/ml were found in eight of 13 patients. Linear regression analysis showed no correlation between cytosol concentrations and serum levels in cervical squamous cell carcinomas ($r = 0.18$). Cytosol concentrations in normal epithelia of the vagina and breast skin were of the same order of magnitude as those in squamous cell carcinomas of the exocervix, vulva, and vagina (Tables 1 and 2).

The median concentrations of SCC antigen in normal squamous epithelia and in squamous cell carcinomas of the exocervix were five to 60-fold higher than those in normal mucosa and adenocarcinomas of the endocervix and 100-fold to 6000-fold higher than those in normal endometrium, cervical stroma, and adenocarcinomas of the endometrium, ovary, and breast (Tables 1 and 2). Antigen concentrations lying above the lowest values measured in normal squamous portio epithelia (194 ng/mg CP) and in cervical squamous cell carcinomas (350 ng/mg CP) were only found in a few specimens of normal mucosa and of adenocarcinomas of the endocervix. However, the highest concentrations measured in normal and malignant lesions of the endocervix did not exceed 1000 ng/mg CP. Elevated SCC antigen levels in serum were only found in one of 12 women with normal glandular epithelia and cervical stroma and in two of 34 patients (6%) with adenocarcinomas.

Patients with primary cervical squamous cell carcinomas had elevated SCC antigen serum levels in 61% of cases. The positivity rate and the serum concentrations

TABLE 2. Squamous Cell Carcinoma Antigen Concentrations in the Cytosol of Squamous Cell Carcinomas and Adenocarcinomas (ng/mg Cell Protein) and in Serum (ng/ml)

	Cytosol			Serum		
	n	Range (ng/mg CP)	Median (ng/mg CP)	Elevated levels (>2.5 ng/ml)	Range (ng/ml)	Median (ng/ml)
Squamous cell carcinoma						
Cervix	15	350–4578	2483	8/13	1.3–61.9	3.7
Vulva	5	1905–6917	2520	0/5	0.5–1.8	1.0
Vagina	2	2124–2450	2287	0/2	1.3–1.4	1.4
Adenocarcinoma						
Cervix	4	167–850	508	1/4	1.3–2.7	1.9
Endometrium	17	1–141	10	0/10	0.5–2.0	1.1
Ovary	10	1–6	1	1/10	0.5–6.0	0.8
Breast	10	1–14	2	0/10	0.6–2.4	1.0

CP: cell protein.

TABLE 3. Incidence of Elevated Squamous Cell Carcinoma Antigen Levels (>2.5 ng/ml) and Median Concentrations in Serum Depending on Tumor Stage and on the Degree of Histologic Differentiation of Primary Cervical Squamous Cell Carcinomas

	Histologic grading							
	G1		G2		G3		Total	
Stage (FIGO)	n	Median (ng/ml)	n	Median (ng/ml)	n	Median (ng/ml)	n	Median (ng/ml)
I	2/4	2.3	5/17	2.2	2/10	1.6	9/31 (29%)	1.9
II	1/1	3.3	14/21	3.6	3/6	3.6	18/28 (64%)	3.5
III	4/4	36.5	17/19	11.7	1/2	21.2	22/25 (88%)	14.5
IV	—	—	6/6	48.1	2/3	6.4	8/9 (89%)	33.1
Total	7/9 (78%)	7.7	42/63 (67%)	4.8	8/21 (38%)	1.8		

FIGO: International Federation of Gynecology and Obstetrics.

measured depended on tumor extent and on the degree of histologic differentiation (Table 3). The incidence of pathologic values increased from 29% in Stage I to 89% in Stage IV, and the median values rose from 1.9 ng/ml to 33.1 ng/ml. Antigen levels in Stage III/IV were significantly different from those in Stages I and II ($P < 0.01$).

Patients with well-differentiated or moderately differentiated carcinomas had pathologic antigen levels in 78% and 67% of cases, respectively. The positivity rate in women with poorly differentiated tumors was only 38%. The serum concentrations in Grade 1 and Grade 2 tumors were significantly higher than those in Grade 3 carcinomas ($P < 0.05$). The median values were highest in Grade 1 (7.7 ng/ml) and lowest in Grade 3 (1.8 ng/ml) tumors. Similar results were found in women with recurrent cervical squamous cell carcinomas. Elevated serum concentrations were measured in all eight patients with Grade 2 tumors, but only in two of five women with Grade 3 carcinomas. However, the values from patients with recurrent Grade 2 tumors were not significantly different from those with Grade 3 carcinomas ($P > 0.05$).

Median SCC antigen concentrations in the cytosol of squamous cell carcinomas were higher in Grade 1 and Grade 2 tumors ($n = 10$, $\tilde{x} = 2522$ ng/mg CP) than in Grade 3 carcinomas ($n = 5$, $\tilde{x} = 970$ ng/mg CP). However, the antigen ranges in both groups were similar (350–4578 and 424–4410 ng/mg CP, respectively). Statistical evaluation of the cytosol concentrations revealed no significant differences ($P > 0.05$).

Discussion

High concentrations of SCC antigen were detected in the cytosol of normal squamous epithelia and of squamous cell carcinomas. The median concentrations were 300-fold to 1500-fold higher than those in normal glandular epithelia and in adenocarcinomas (Fig. 1). The highest median value was found in normal squamous portio epithelia exceeding that of cervical squamous cell carcinomas by a factor of 2.3. In glandular epithelia the antigen concentrations were highest in normal mucosa and in carcinomas of the endocervix. Some values even overlapped with the lowest concentrations measured in squamous epithelia. This fact could be attributed to the contamination of some endocervical tissue specimen with small parts of adjacent squamous epithelia. Entirely pure tissue preparations of normal glandular epithelia and adenocarcinomas of the endometrium, ovary and breast without squamous parts contained only very low antigen concentrations.

Similar results were reported by Morioka[21] who found 10-fold to 50-fold higher TA-4 concentrations in normal and malignant lesions of squamous portio epithelia than in normal endocervical mucosa. Neither cervical stroma and normal endometrium nor adenocarcinomas of the endocervix, endometrium, and ovary had measurable TA-4 activities. In contrast to the results of the current study, the mean TA-4 concentrations measured in cervical squamous cell carcinomas were five-fold higher than those in normal squamous portio epithelia. According to the results of flow cytometric analysis in cervical squamous cells, the TA-4 content in cancer cells was indeed eight times greater than in normal cells.[22]

However, in the esophagus significantly higher SCC antigen concentrations were measured in normal squamous epithelia than in squamous cell carcinomas.[18] The reason for the differing TA-4 and SCC antigen ratios in these studies may be the heterogeneity of the glycoprotein TA-4 which consists of 14 subfractions with isoelectric points (IEP) ranging from 5.44 to 6.62.[4] The SCC antigen is a nearly pure protein with a carbohydrate content of less than 0.6% and has the most neutral IEP (6.62) of all subfractions.[4,12] Kato *et al.*[23] demonstrated in an earlier study that neutral TA-4 (IEP, 6.3–6.6) is the predominant

fraction in nonmalignant squamous epithelia, whereas acidic TA-4 (IEP, 5.9–6.2) is mainly present in the tissue and serum of patients with cervical squamous cell carcinomas. Since it is not clear which subfraction of TA-4 was measured in the studies of Morioka[21] and Sasaki *et al.*,[22] tissue measurements of TA-4 and SCC antigen cannot be directly compared.

The results of TA-4 and SCC antigen determinations in the cytosol are in agreement with the data of an immunohistochemical study by Ueda *et al.*,[3] who detected TA-4 in all normal squamous portio epithelia, in most of cervical squamous cell carcinomas and in a few normal columnar epithelia and adenocarcinomas of the endocervix, but not in normal glandular epithelia and in carcinomas of the endometrium.

Despite the high SCC antigen concentrations in the cytosol of normal squamous epithelia, serum levels were nearly always within the normal range. Women with cervical squamous cell carcinomas had lower tissue concentrations, but higher serum values of SCC antigen. According to our own results and to the data of Morioka,[21] there was no correlation between cytosol concentrations and serum levels of SCC antigen or TA-4 in cervical squamous cell carcinomas. The median serum concentrations and the incidence of elevated serum levels increased with tumor extent. These findings indicate that the release of SCC antigen into the circulation depends on infiltrative tumor growth and on tumor mass, but not on the local antigen content in the tissue.

Analogous findings have been reported for carcinoembryonic antigen (CEA). There was no correlation between plasma and tumor concentrations of CEA in carcinomas of the liver, lung, gastrointestinal, and female genital tract.[24,25] Van Nagell *et al.*[25] supposed that plasma CEA reflects the total tumor burden (*i.e.*, tumor CEA concentration × tumor mass) rather than the tumor CEA concentration alone.

However, these conclusions cannot explain the fact that 10% to 20% of patients with cervical squamous cell carcinomas Stages III and IV have normal SCC antigen serum levels. Apparently, additional factors influence the release of the marker, one of those being the differentiation of the carcinomas. The incidence of elevated SCC antigen levels and the median serum values were higher in patients with well-differentiated and moderately differentiated cervical squamous cell carcinomas than in women with poorly differentiated tumors. Median cytosol concentrations in Grade 1 and Grade 2 carcinomas were higher than in Grade 3 tumors. Similar data were found in squamous cell carcinomas of the esophagus.[18] These findings are also confirmed by the results of immunohistochemical studies. In the normal squamous epithelium of the cervix, TA-4 was detectable only in differentiated squamous cells of the intermediate layer, and not in the immature cells of the basal layer.[3,12] In cervical squamous cell carcinomas, TA-4 was positive in more differentiated tumors such as large cell keratinizing and large cell nonkeratinizing carcinomas, but never in small cell nonkeratinizing carcinomas.[3,10]

The results of our investigation show that SCC antigen is not tumor specific. High quantities are present not only in squamous cell carcinomas, but also in the cytoplasm of normal squamous epithelia. The release of the antigen into the circulation depends on infiltrative growth and the mass of the tumors rather than on the local tissue content. Apparently, SCC antigen is a marker of cell differentiation for squamous cells. Further characterization of SCC antigen requires simultaneous histologic examinations, immunohistochemical studies, and measurement of concentrations in the cytosol and serum in a large number of patients with cervical squamous cell carcinomas. Biochemical studies are needed to elucidate the differences between TA-4, SCC antigen, and other subfractions.

REFERENCES

1. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer* 1977; 40:1621–1628.
2. Kato H, Morioka H, Aramaki S, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cell Mol Biol* 1979; 25:51–56.
3. Ueda G, Inoue Y, Yamasaki M *et al.* Immunohistochemical demonstration of tumor antigen TA-4 in gynecologic tumors. *Int J Gynecol Pathol* 1984; 3:291–298.
4. Ikeda I. Two-site radioimmunometric (sandwich) assay of SCC antigen using monoclonal antibodies. In: Kato H, de Bruijn HWA, Ebert W, Herberman RB, Johnson JT, eds. Squamous Cell Carcinoma Antigen in the Management of Squamous Cell Carcinoma. Princeton: Excerpta Medica, 1987; 215–226.
5. Kato H, Miyauchi F, Morioka H, Fujino T, Torigoe T. Tumor antigen of human cervical squamous cell carcinoma: Correlation of circulating levels with disease progress. *Cancer* 1979; 43:585–590.
6. Kato H, Morioka H, Tsutsui H, Aramaki S, Torigoe T. Value of tumor-antigen (TA-4) of squamous cell carcinoma in predicting the extent of cervical cancer. *Cancer* 1982; 50:1294–1296.
7. Kato H, Morioka H, Aramaki S, Tamai K, Torigoe T. Prognostic significance of the tumor antigen TA-4 in squamous cell carcinoma of the uterine cervix. *Am J Obstet Gynecol* 1983; 145:350–354.
8. Kato H, Tamai K, Morioka H, Nagai M, Nagaya T, Torigoe T. Tumor antigen TA-4 in the detection of recurrence in cervical squamous cell carcinoma. *Cancer* 1984; 54:1544–1546.
9. Kato H, Tamai K, Nagaya T, Nagai M, Torigoe T. The use of tumor antigen TA-4 for the management of squamous cell carcinoma. *Cancer Detect Prev* 1985; 8:155–159.
10. Maruo T, Shibata K, Kimura A, Hoshina M, Mochizuki M. Tumor-associated antigen, TA-4, in the monitoring of the effects of therapy for squamous cell carcinoma of the uterine cervix. *Cancer* 1985; 56:302–308.
11. Obata Y, Tadokoro M, Kazabo S *et al.* Basic evaluation of measurement of the serum level of squamous cell carcinoma-related antigen (SCC) and its value following irradiation of cancer of the uterine cervix. *Gan No Rinsho* 1987; 33:60–64.

12. Kato H, Morioka H, Hashimoto K *et al.* SCC antigen and its clinical applications. In: Kato H, de Bruijn HWA, Ebert W, Herberman RB, Johnson JT, eds. Squamous Cell Carcinoma Antigen in the Management of Squamous Cell Carcinoma. Princeton: Excerpta Medica, 1987; 1–14.

13. Crombach G, Würz H, Bolte A. Determination of SCC antigen in the serum of patients with carcinoma of the cervix uteri. *Geburtshilfe Frauenheilkd* 1987; 47:439–445.

14. Senekjian EK, Young YM, Weiser PA, Spencer CE, Magic SE, Herbst AL. An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma. *Am J Obstet Gynecol* 1987; 157: 433–439.

15. Oishi T, Maruo T, Yamasaki M, Mochizuki M. Prediction of the recurrence of squamous cell carcinoma of the uterine cervix by monitoring serum TA-4. *Nippon Sanka Fujinka Gakkai Zasshi* 1987; 39: 799–806.

16. Ebert W, Johnson JT. Tumormarkers in the Management of Squamous Cell Carcinoma of the Head, Neck and Lung. Princeton: Excerpta Medica, 1987.

17. Masuoka T, Matueda Y, Dokawa H, Watanabe K, Mimoto S. The measurement of SCC antigen in squamous cell carcinoma of the lung. *Gan No Rinsho* 1985; 31:914–918.

18. Gion M, Mione R, Dittadi R, Bruscagnin G. SCC antigen in patients with esophageal carcinoma. In: Kato H, de Bruijn HWA, Ebert W, Herberman RB, Johnson JT, eds. Squamous Cell Carcinoma Antigen in the Management of Squamous Cell Carcinoma. Princeton: Excerpta Medica, 1987; 130–141.

19. Lowry DH, Rosebrough NJ, Farr AL, Fandall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193:265–275.

20. Ferenczy A. Carcinoma and other malignant tumors of the cervix. In: Blaustein A, ed. Pathology of the Female Genital Tract, ed. 2. New York: Springer, 1982; 184–222.

21. Morioka H. Tumor antigen (TA-4) of squamous cell carcinoma: Its tissue distribution and its relationship to serum TA-4 concentrations. *Asia Oceania J Obstet Gynecol* 1980; 6:91–97.

22. Sasaki K, Nagai M, Kato H, Torigoe T, Nagamine Y, Takahashi M. Flow cytometric analysis of tumor antigen TA-4 in cervical squamous cells. *Gann* 1984; 75:703–706.

23. Kato H, Nagaya T, Torigoe T. Heterogeneity of a tumor antigen TA-4 of squamous cell carcinoma in relation to its appearance in the circulation. *Gann* 1984; 75:433–435.

24. Khoo SK, Warner NL, Lie JT. Carcinoembryonic antigen activity of tissue extracts: A quantitative study of malignant and benign neoplasms, cirrhotic liver, normal adult and fetal organs. *Int J Cancer* 1973; 11:681–687.

25. Van Nagell JR, Donaldson ES, Wood EG, Sharkey RM, Goldenberg DM. The prognostic significance of carcinoembryonic antigen in the plasma and tumors of patients with endometrial adenocarcinoma. *Am J Obstet Gynecol* 1977; 128:308–313.